RESEARCH ARTICLE

Noval Antimicrobial Protein Fabrics using Pharmaceutical Waste

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ABSTRACT: This study focuses on smart functionalization of protein fabric preparation by using the synergistic antimicrobial agents like expired antibiotic drug amoxicillin. Protein fabrics like wool and silk containing different groups of amino acids, has been pre-treated with expired drug amoxicillin and chitosan at various concentrations (1%, 2%, 3% OWF) (to impart antimicrobial/antibacterial properties to fabric). The modified wool or silk fabric was then dyed with four different acid dye using exhaust method in a Rota dyer. The colour strength value and K/S value were evaluated by using spectrophotometer and the antimicrobial activity is evaluated by using the AATCC 147 test method. The above dyed samples have shown good washing, light fastness properties with high depth of colour value. The pre treated wool and silk fabrics were coated with Amoxicillin along with chitosan have shown an excellent antimicrobial activity. Amoxicillin attaches to the cell wall of susceptible bacteria and inhibit the growth of bacteria results in their death. The antibiotic coating is effective against streptococci, pneumococci, enterococci, *Haemophilusinfluenzae*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella*, *Borreliaburgdorferi*, and *Helicobacter pylori*. The presence of chitosan is further enhanced both by increasing the rate of dye uptake as well as inhibit the growth of micro organism

KEYWORDS: Amoxicillin, Chitosan, Antimicrobial, Acid Dye, Protein Fabric

https://doi.org/10.29294/IJASE.6.1.2019.1226-1232

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1. INTRODUCTION

Amoxicillin is beneficial for the treatment of a number of bacterial infections. It may also be used for strep throat, pneumonia, skin infections, and urinary tract infections among others. It is taken by mouth, or less commonly by injection [1, 2].

In 1958, antibiotic drug like amoxicillin was discovered and in 1972 it was used for medical purpose.[3,4]. Recommended by World Health Organization's List of crucial Medicines which are safe to use and efficient in their action [5]. It is a moderate-spectrum, bacteriolytic, β-lactam antibiotic in the amino-penicillin family used to treat susceptible Gram-positive and Gram-negative bacteria.In general, Streptococcus, Bacillus subtilis, are susceptible to amoxicillin, whereas Citrobacter, Klebsiella and Pseudomonas aeruginosa are resistant to it. Some E. coli and most clinical strains of Staphylococcus aureus have existed resistance to amoxicillin to varying degrees [6-12]. Chitosan is a modified carbohydrate derived from chitin component of the shells of crustacean, such as crabs, shrimps and prawns. It is a natural bio-polymer with reactive hydroxyl and amino groups so it is amphoteric in nature and also it is biocompatible, biodegradable, fungicide, and nontoxic [13].

1.1 *Natural fibers*

Natural fibers like wool and silk fibres are called as Protein fibers since they contain a large group of amino acids and their derivatives. Wool is obtained from animals like sheep, alpaca, camel, cashmere, goat etc. and silk fiber is extruded in the filament form from insects like silkworm caterpillar. The main protein in wool is keratin. In silk fibers serecin and fibroin are dominant compounds. All protein are made up of various amino acids to form repeating polyamide units, wool possess 16 different types of amino acids silk possess 12 types of amino acids, and hence protein fibers exhibits amphoteric property. Besdes the presence of these amino acids in protein fibers, makes it highly susceptible to attacks by microorganisms and thus degrade the fiber. To prevent this microbial degradation of these fibers, treatment with antimicrobial agents are a must either as a finish or as a pretreatment applied on these textile substrates [14-17].

Due to larger surface area and capability to retain moisture from the surrounding environment, natural fibres like cellulosic fibers as well as protein fibers provide the suitable platform to growth of microorganism [18]. The microbial growth on to the fabric cause undesirable effects like unpleasant odour, stain or discoloration of the fabric reduction in tensile strength and tear strength. Infestation by micro-organism causes cross infection by pathogens and development odour where the fabric is worn next to human skin contaminate the environment [19]. Various microorganisms including bacteria, Fungi or insects are responsible for infections and allergic reactions [20].

Thus the major problem in tropical countries where humidity and temperatures are high growth of microorganisms, insects and fungi is inevitable on these natural fibres. In addition, the staining and loss of

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Received: 19.06.2019 **Accepted:** 10.08.2019 **Published on**: 28.08.2019

the performance properties of textile substrates are the results of microbial attack. Thus to prevent the microbial degradation of proteinfiber and cellulosic fiber is a giant problem and has become a growing concern in textile industry,

1.2 Anti-Microbial Technology in Textile Industry

The textile industry has tried to overcome the above problem especially in wool and silk and also on cotton by adapting various types of Functional finishes, and speciality chemicals to impart anti microbial and anti insecticidal and also antifungal property to these substrates. Novel finishing of fabrics has been appreciated by a more discerning and demanding consumer market. The most important step in textile production is textile wet processing which imparts value addition in textiles through improvement in aesthetics, comfort and functional properties [21].

Antimicrobial textiles which improve functionality find a variety of applications in healthcare and hygiene products especially the garment worn close to the skin, and several other medical applications or medical devices for infection control and as barrier material [22-23].The major application of antimicrobial textiles is in the medical field and hence also known as medical textiles to help maintain sterile or hygienic environments. Antimicrobial finishing for textile materials is compulsory to fulfil the following objectives, namely to avoid cross infection by pathogenic micro-organisms; to control the infestation by microorganisms; to stop metabolism in microbes in order to reduce the formation odour; to protect the textile products from staining, discolouration and quality deterioration [24].

1.3 Requirements for Antimicrobial Finish

Clothing and textile materials especially wool or silk garments are more susceptible to wear and tear [25].Followings are the some essential conditions to obtain maximum benefits of the antimicrobial finish on textile substrates: Anti-microbial finish should possess good shelf life and have good wash and light fastness and also fast to dry cleaning and hot pressing, should possess selective activity to undesirable microorganisms. The antimicrobial finished fabric should be harmless to the users, manufacturer and the environment. It should compatible with the requirements of regulating agencies. Easy method of application, non toxic and also it should not deteriorate the fabric quality [19].

1.4 Benefits of Antimicrobial Textiles

Novel technologies in antimicrobial finishing are successfully imparted in woven and non-woven sector especially in medical textiles. Antimicrobial finished textile will also distribute the purpose alone or in blends with other fibres. Bioactive fibre is a modified form of the antimicrobial finish. Niche applications for bioactive fabrics being in the medical &defence industries, for e.g. drug, bandages or protective clothing with extremely highly sensitive cellular sensors. "Bio-active" substrates belong to "Functional" variety of products, i.e. to textiles, which, in addition to their basic functions, claim to take care of the hygiene and health of the consumer. [26-27].Bioactive fibres contains sanitary materials, pharmaceutical industry , dressing materials, surgical threads, materials for filtration of gases and liquids, air conditioning and ventilation, constructional materials, special materials for food industry, footwear industry, clothing industry, automotive industry etc. [28-32].

2. EXPERIMENTAL

2.1 Material Used

Substrate- Silk fabric (100 GSM) and Wool Fabric (250 GSM) was obtained from Neelkanth Fabrics Private Limited, Mumbai. Dye- Sandolan Red Brown, Sandolan MBlack, ChhataraABrown-97, Sandolan Rhodamine E2GLI and chemicals like Ammonium Acetate, Acetic Acid, Non-Ionic soap were obtained from ICT, Mumbai. Chitosan was purchased from Marshall Marine Products ltd,Tamilnadu. Amoxicillin (Almox 500 mg capsule) obtained from Alkem Laboratories Ltd.

2.2 Methodology

Scoured and bleached wool and silk fabrics were pretreated with various concentrations of amoxicillin drug(one single capsule of Almox 500 contain only 500 mg amoxicillin without any other salts) (1%, 2% etc) and chitosan bio-polymer (1gpl, 2 gpletc) in rota dyer machine for 30 mins at 80^{0}C temperature, The pre-treated protein fabrics and the untreated wool and silk fabrics were dyed by the exhaust method with acid dyes, in a rota dyer (ROSSARI).The percentage shade used for acid dyeing on wool and silk fabric was 1% for all above dyes. The soaping is given with non-ionic soap at 80^0 C for 30 mins M:L::1:40 followed by washing in hot water and then washing in cold water. After washing the samples are dried at room temperature and evaluated for their performance properties

2.3 Antimicrobial activity Test (AATCC 147 method)

This method is used to determine qualitative activity analysis of antimicrobial agent on finished fabric. It is also called Parallel Streak method [33-34]. Finished textile fabric was cut into 25-50 mm in size and test organism used for this Antimicrobial activity Test was Gram positive organism (*Staphylococcus aureus*) and gram negative organism (*E.coli*).According to AATCC 147 method, an inoculum was made as 1.0±0.1 ml of a 24 h culture in nutrient broth (NB) was moved into 9.0± 0.1 ml of sterile distilled water. By using sterile an inoculating loop, 5 consecutive streaks of the diluted inoculum were made over a standard Petri dish containing nutrient agar (NA) media, without refilling the loop. Treated and standard textile samples were placed over the streaks; make certain contact with the nutrient agar surface. The Petri dishes were incubated for 24 h at 37±2°C (AATCC 147 2004) [35]. After completing the incubation, Petri dishes were removed from the incubator and the anti-bacterial activity was assessed by zone of inhibition. Zone of inhibition is circular area around the treated sample in which microorganism's colonies do not grow [36].

2.4 Characterization

UV Absorption

The above acid dyed wool and silk fabrics were evaluated by using the UV spectrophotometer for the fixation of dye into the fabric through UV spectra. UV spectrophotometer was used in the quantitative analysis of concentrations of the absorber in the solutions of metal ions and highly conjugated organic compounds. The antimicrobial activity is evaluated by using the AATCC 147 test method. The difference is calculated between the conventional dyeing and the dyeing in presence of amoxicillin or chitosan. The fixation of the dye is observed through the following results [37].

K/S Value

It is a specification of colorimeter, which can be point out to determine the depth of colour of the dyed samples.Spectra flash, Computer Colour matching system supplied by Data colour international, U.S.A was used for the evaluating the colour depth of above dyed sample [38].Kulbeka Munk K/S function which is given by:

$$K/S= [\{(1-R^2/2R)\}]$$

Where,

"R" is the reflectance at complete opacity."K" is absorption coefficient. "S" is the scattering coefficient.

3. RESULT AND DISCUSSION

Protein fabrics like wool and silk which are made up of different amino acids when exposed to moisture for long periods, the fabric provides enough nutrients for bacterial growth. So these fibers can be easily attacked by the microorganisms. The main objective of process is to impart anti-microbial property to the protein substrates to prevent microbial degradation of fabric. At the outset, conventional dyeing was carried outon wool and silk at 1% shade using acid dyes.In the next set, wool and silk fabrics were pretreated with chitosan, amoxicillin and a proportionate mixture of chitosan and amoxicillin respectively and then they were dyed conventionally as above with acid dyes at 1% shade. The dyed fabrics were evaluated for their performance properties and the results are given in the following tables

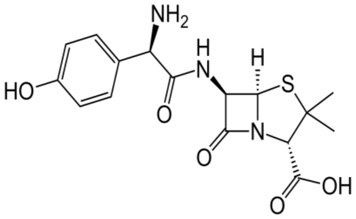


Figure 1 Amoxicillin

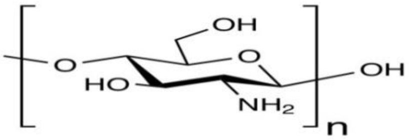


Figure 2 Chitosan

Table 1 Performance properties of Acid dyed wool fabric

Dye Name	Dyed Sample	K/S	UV absorption	Wash Fastness	Light Fastness
Sandolan Red Brown (1% shade)	Std Dyed wool	3.74	2.05	4-5	6-7
	Pre-treated with 1% amoxicillin	7.50	2.78	4-5	6-7
	Pre-treated with 2 gpl chitosan	5.60	2.58	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	8.70	2.70	4-5	6-7
Sandolan M Black	Std Dyed wool	2.40	2.15	4-5	6-7
	Pre-treated with 1% amoxicillin	4.68	2.89	4-5	6-7
	Pre-treated with 2 gpl chitosan	3.70	2.75	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	4.75	2.80	4-5	6-7
Chhatara A Brown-97	Std Dyed wool	10.58	2.90	4-5	6-7
	Pre-treated with 1% amoxicillin	13.86	3.36	4-5	6-7
	Pre-treated with 2 gpl chitosan	11.26	3.12	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	15.74	3.70	4-5	6-7
SandolanRhodamine E2GLI	Std Dyed wool	1.36	2.68	4-5	6-7
	Pre-treated with 1% amoxicillin	2.47	2.96	4-5	6-7
	Pre-treated with 2 gpl chitosan	1.71	2.89	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	2.18	2.78	4-5	6-7

Table 2 Performance properties of Acid dyed Silk fabric

Dye Name	Dyed Sample	K/S	UV absorption	Wash Fastness	Light Fastness
Sandolan Red Brown (1% shade)	Std Dyed Silk	3.15	1.98	4-5	6-7
	Pre-treated with 1% amoxicillin	6.74	2.28	4-5	6-7
	Pre-treated with 2 gpl chitosan	5.48	2.38	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	6.12	2.50	4-5	6-7
Sandolan M Black	Std Dyed Silkl	2.08	2.15	4-5	6-7
	Pre-treated with 1% amoxicillin	2.38	2.69	4-5	6-7
	Pre-treated with 2 gpl chitosan	2.18	2.75	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	2.48	2.60	4-5	6-7
Chhatara A Brown-97	Std Dyed Silk	8.56	2.62	4-5	6-7
	Pretreated with 1% amoxicillin	11.86	2.36	4-5	6-7
	Pre-treated with 2 gpl chitosan	10.26	3.12	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	12.74	2.70	4-5	6-7
SandolanR hodamine E2GLI	Std Dyed Silk	1.36	2.68	4-5	6-7
	Pre-treated with 1% amoxicillin	2.48	2.96	4-5	6-7
	Pre-treated with 2 gpl chitosan	1.74	2.79	4-5	6-7
	Pre-treated with 1% amoxicillin & 2gpl chitosan	2.18	2.88	4-5	6-7

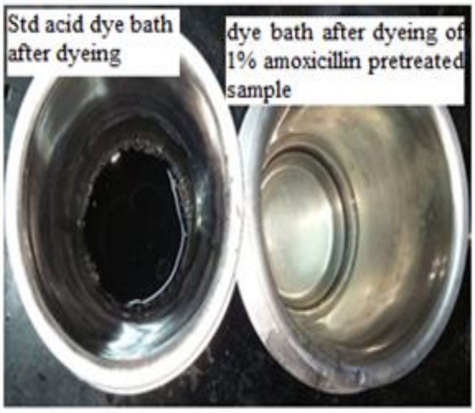

Std acid dye bath after dyeing
dye bath after dyeing of 1% amoxicillin pretreated sample

Figure 3Sandolan M Black dye bath

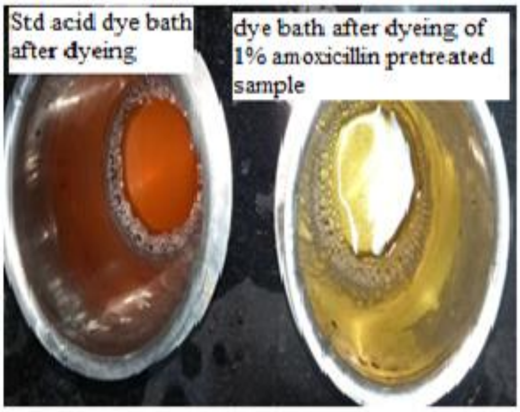
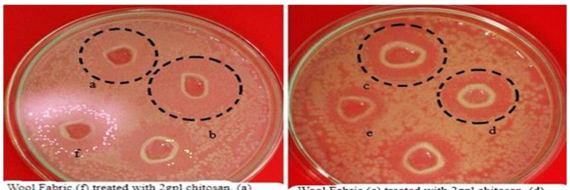

Std acid dye bath after dyeing
dye bath after dyeing of 1% amoxicillin pretreated sample

Figure 4SandolanRhodamine E2GLI dye bath

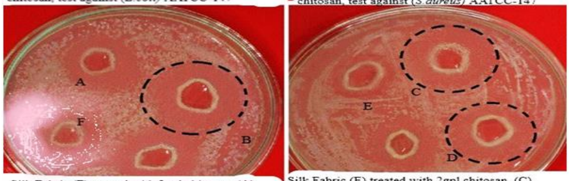
Table 1 Evaluation of anti-microbial activity of wool & silk fabric treated with chitosan /amoxicillin

Dye Name	Dyed Sample	Inhibition Zone (mm)		Dyed Sample	Inhibition Zone (mm)	
		With *E. coli*	With S. aureus		With *E. coli*	With S. aureus
Sandolan Red Brown (1% shade)	Std Dyed wool	0	0	Std Dyed Silk	0	0
	Pre-treated with 1% amoxicillin	34	32	Pre-treated with 1% amoxicillin	33	30
	Pre-treated with 2 gplchitosan	12	10	Pre-treated with 2 gpl chitosan	12	10
	Pre-treated with 1% amoxicillin & 2gpl chitosan	38	34	Pre-treated with 1% amoxicillin & 2gpl chitosan	36	32
Sandolan M Black	Std Dyed wool	0	0	Std Dyed Silk	0	0
	Pre-treated with 1% amoxicillin	34	32	Pre-treated with 1% amoxicillin	33	30
	Pre-treated with 2 gpl chitosan	12	10	Pre-treated with 2 gpl chitosan	12	10
	Pre-treated with 1% amoxicillin & 2gpl chitosan	38	34	Pre-treated with 1% amoxicillin & 2gpl chitosan	36	32
Chhatara A Brown-97	Std Dyed wool	0	0	Std Dyed Silk	0	0
	Pre-treated with 1% amoxicillin	34	32	Pre-treated with 1% amoxicillin	33	30
	Pre-treated with 2 gpl chitosan	12	10	Pre-treated with 2 gpl chitosan	12	10
	Pre-treated with 1% amoxicillin & 2gpl chitosan	38	34	Pre-treated with 1% amoxicillin & 2gpl chitosan	36	32
SandolanRhodamine E2GLI	Std Dyed wool	0	0	Std Dyed Silk	0	0
	Pre-treated with 1% amoxicillin	34	32	Pre-treated with 1% amoxicillin	33	30
	Pre-treated with 2 gpl chitosan	12	10	Pre-treated with 2 gpl chitosan	12	10
	Pre-treated with 1% amoxicillin & 2gpl chitosan	38	34	Pre-treated with 1% amoxicillin & 2gpl chitosan	36	32



Wool Fabric,(f) treated with 2gpl chitosan, (a) treated with 1% amoxy & (b) 1% amoxy+2gpl chitosan, test against (*E.coli*) AATCC-147

Wool Fabric,(e) treated with 2gpl chitosan, (d) treated with 1% amoxy & (c) 1% amoxy+2gpl chitosan, test against (*S.aureus*) AATCC-147



Silk Fabric,(F) treated with 2gpl chitosan, (A) treated with 1% amoxy & (B) 1% amoxy+2gpl chitosan, test against (*E.coli*) AATCC-147

Silk Fabric,(E) treated with 2gpl chitosan, (C) treated with 1% amoxy & (D) 1% amoxy+2gpl chitosan, test against (*S.aureus*) AATCC-147

Figure 5 Evaluation of antimicrobial activity of treated samples against Gram (+) & Gram (-) bacteria

As observed in Table 1 and Table 2, 1% amoxicillin pre-treated wool and silk fabric are showing higher values of K/S and UV absorbance values compared to standard conventionally acid dyed wool and silk sample. This may be due to presence of azo group or amine group in amoxicillin it increase the depth of colour and also increases the 100% dye absorption from the dye bath (Figure 3&4). When protein fabrics(wool and silk) were pre-treated with 2 gpl chitosan and then dyed with various acid dyes, it increases the depth of colour, compared to standard dyed sample, but slightly less depth compared to amoxicillin pre-treated samples. When wool and silk fabric were pre-treated with both amoxicillin and chitosan solution and then dyed with various acid dyes then it exhibits good depth of colour, higher UV absorbance with higher antimicrobial activity against gram positive and gram negative bacteria. The exhaustion of the dye bath is nearly 90% leading to very less colour in the effluent. (Figures 3&4)

Chitosan is a natural biopolymer and has good absorption properties because of its structure similar to cyclodextrene and hence the colour value increases and absorption of amoxicillin increases and a synergism is observed, besides, chitosan possess anti microbial properties too. On the whole chitosan increases, the percentage add on, due to increased rate of dye uptake and hence increased uptake of amoxicillin and better colour value and antimicrobial property

On comparing the antimicrobial properties imparted to the fabric by using amoxicillin, chitosan biopolymer as well as mixture of the both antimicrobial agents as seen in Table 3 and Figure 5, for both wool and silk fabrics amoxicillin gives maximum antimicrobial values or maximum zone of inhibition (34mm) followed by chitosan which give 12 mm of zone of inhibition but surprisingly the mixture of 1% amoxicillin and 2 gpl chitosan gives the maximum antimicrobial values that one is 38 mm of zone of inhibition as seen in the table 3. Thus it is observed that amoxicillin in presence of chitosan gives good antimicrobial properties on wool, silk fabric.

4. CONCLUSION

Attack by micro-organism is inevitable on wool and silk fibres causing loss in strength, shelf life and marketability. Value added finishing and treatment with antimicrobial agents, seems to be the solution for the above problem and hence a lot of research activity is being carried out in this field, Here, an attempt has been made to pretreat the protein fabric with a pharmaceutical drug namely amoxicillin and biopolymer (Chitosan) to increase shelf-life of fabrics by imparting antimicrobial property to the wool and silk fabrics. This is a successful venture to prepare asmart hygienic textile fabric which is anti microbial and well suited for medical textiles. It is also suggested the organised collection of waste drugs either from hospitals; household, medical stores or companies manufacturing the drugs can have a profitable enterprise in textile processing.

ACKNOWLEDGEMENT

The Authors are thankful to the Department of Fiber and Textile Processing Technology, Institute of Chemical Technology, Matunga, Mumbai for providing infrastructural facilities and Department of Science and Technology (DST) India for providing financial support for this research work.

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